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16. Abstract

The authors investigated the numbers of erythrocytes and percentage of hemoglobin in plasma in 20 men after subjection to cold showers and hard work. Erythrocytes dropped in numbers and hemoglobin was found in plasma for up to an hour.

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MYOGENIC CAUSES OF HEMOLYSIS

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Up to now opinions have explained the process of erythrocyte reduction by the activity of the reticuloendothelial system, concentrated mainly in the spleen. Is this the only way of removing red blood cells from the blood stream? /33*

Buerger [3] observed a drop in the number of red cells after cooling the body to the shivering point, while Arnold [1] found it after hard physical exertion. This quickly compensated drop further stresses the need to consider the role of the skeletal muscles in the process of lowering the number of red blood cells.

The fact that hemoglobin always appears in the plasma after thorough chilling of the body prompted us to seek an explanation of the cause of this phenomenon [Bula]. In this regard we began research to demonstrate whether there is a drop in erythrocytes after chilling, and also after physical work, and what its cause might be.

Methodology

Our research included twenty men from 19 to 22 years old, divided into two groups. The subjects in the first group were subjected to intense chilling under a shower with water at about 14° C for ten to fifteen minutes until definite shivering occurred.

The subjects of the second group were given physical work of a nature requiring strength for a period of 30 minutes (training in weightlifting). Blood was drawn before freezing and exertion, immediately after exertion, and an hour later. Blood was drawn in the following way: a needle was inserted into the cubital vein and drops of flowing blood were drained into a clean, dry test tube and centrifuged at a speed of 3500 rpm. Blood was also taken from the

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*Numbers in the margin indicate pagination in the foreign text.

same vein to determine the number of erythrocytes, their resistance, and the hemoglobin content in the blood. The number of erythrocytes per mm^3 was computed in the way generally accepted with the use of a Thom-Zeiss table. The cells were counted three times in each examination and the mean value was taken for further statistical calculations. The erythrocyte resistance was determined in relation to an aqueous NaCl solution in concentrations from 0 to 1 percent, /35 up to 0.05 percent. Blood hemoglobin was determined with Sahli's method, and plasma hemoglobin by the method given by Masiak, Grodzienski and Sierawski [4]. This method is based on the colorimetric determination of hemoglobin with a benzidine reagent. The iron contained in the hemoglobin catalyzes a benzidine oxidation reaction through H_2O_2 (Baush and Lomb Spectronik 20) with a wavelength of 515 mμ. The result is read from a standard curve pattern. The results obtained were statistically elaborated by taking the total number of subjects as Spermann's "small sample".

Table I. Hemoglobin Content in Erythrocytes and in Blood Plasma, Changes in the Number of Erythrocytes, and Their Resistance at Rest and After Chilling and Physical Exertion. /34

		At rest	After chilling		At rest	After physical exertion	
			After 15 min	After 60 min		After 15 min	After 60 min
% Hg in venous blood	$\bar{X}$	95	95	95	93	93	93
	s	5	4,2	4,5	5,1	5,2	5,1
Mg % Hg in plasma	$\bar{X}$	0	16,10	13,50	0	13,33	8,99
	s	0	7,55	12,04	0	12,56	6,78
No. of erythrocytes per mm^3 of blood	$\bar{X}$	4 635 000	4 329 000	4 594 000	4 422 000	4 233 000	4 927 000
	s	710 000	889 000	509 000	651 000	478 000	478 000
Concentration of NaCl (%) causing corpuscle hemolysis	$\bar{X}$	0,45—0,37	0,40—0,32	—	0,45—0,35	0,42—0,28	—
	s	0,13	0,08	—	0,10	0,14	—

Key: X = Arithmetic Mean; s = Standard Deviation.

Note: Commas indicate decimal points.

Results and Discussion

Hemoglobin appeared in the plasma of all subjects immediately after chilling. It was at a substantial level and varied within the limits of 7 to 27 mg percent with a mean of 16.10 mg percent. Hemoglobin was still found in the plasma after an hour, but its level was reduced and varied within the limits of 0 to 35 mg percent with a mean of 13.50 mg percent. The great difference in hemoglobin concentration an hour after chilling was remarkable. In two cases this level was reduced to 0 (zero), in one case rose to 35 mg percent, a level not found immediately after chilling.

In the majority of cases the plasma hemoglobin drops, as demonstrated by the mean arithmetical value. However, differences in the plasma hemoglobin level immediately after chilling and one hour later are not statistically valid. This fact is proven by the various individual rates of removing hemoglobin from the plasma.

Just as after chilling, hemoglobin also appears in the plasma after physical exertion. Immediately after exertion the hemoglobin content varied from 10 to 46 mg percent, with the mean value amounting to 13.33 mg percent. This level was already diminished one hour after exertion and varied from 0 to 17 mg percent with a mean of 8.99 mg percent. Just as after chilling, the difference in the hemoglobin level immediately after work and one hour later was statistically invalid.

A comparison of standard deviations shows that dispersion (sigma) of the percentage content of hemoglobin in the plasma one hour after chilling was still twice as great as immediately after chilling. We find a reverse situation after physical effort, with dispersion diminishing an hour after work. This result stems from the fact that hemoglobin removal is somewhat more rapid after physical work and progresses in a more uniform way.

After chilling the amount of erythrocytes also drops, an average of 300,000 per mm^3, and returns to almost the initial position after an hour. Changes in the number of erythrocytes are associated with differences in dispersion, which does not differ essentially before and after chilling, but is considerably less an hour afterward. This shows that the increase in the number of erythrocytes after chilling progresses at less variable average rates. Similar changes were found in the number of erythrocytes after physical exertion, where the drop in erythrocytes immediately after physical exertion amounted to 200,000 per mm^3. After an hour the number of corpuscles had increased by 500,000 per mm^3. In contradistiction to chilling, the dispersion was not subject to change after an hour had passed.

The drop in the number of erythrocytes immediately after chilling and physical effort affected all the subjects and exceeded the limits of probable error. Therefore this drop in the number of erythrocytes cannot be explained as a computational error (laboratory error), although it is invalid. Only the difference in the erythrocyte increase between the number found immediately after exertion and an hour later is statistically valid. /36

Red blood corpuscle resistance research show that complete hemolysis before work and chilling appears in concentrations from 0.45 to 0.33 percent NaCl, while after work and chilling it appears in concentrations from 0.40 to 0.30 NaCl. Comparison reveals that the corpuscles exhibit more resistance after chilling and physical exertion.

Total blood hemoglobin is not subject to change after either chilling or physical exertion. Nor do standard deviations show any expressed variations before and after work and chilling.

Discussion

The presence of hemoglobin in the plasma, the drop in the number of erythrocytes, the increase in their resistance and the unchanged level of the hemoglobin in venous blood after chilling and physical work all point to a similar causative mechanism. The presence of hemoglobin in the plasma with a simultaneous drop in the number of erythrocytes implies that the process of destruction does not occur exclusively in the reticuloendothelial system where the red blood corpuscle is treated enzymatically, the porphin ring of the heme broken down, and iron removed. In the case of heme iron alone, if hemoglobin had penetrated the blood stream after the decrease in red corpuscles, we could not have helped detecting it in the method we used. Moreover, the destruction of red corpuscles in the reticuloendothelial system is reduced during strain on the sympathetic system occasioned by chilling and physical work, as well as by other causes.

It could be thought that the hemoglobin appeared after physical exertion as a result of chemical factors. It is a well-known fact that under such circumstances the pH of the blood and the supply of alkaline reserves diminish as a result of the increase in the concentrations of various compounds with an acidic reaction. Such modifications have been ascertained only after intensive and prolonged physical exertion, and cannot be a causative factor in our research because brief exertion, and especially chilling, could not cause such a concentration of acidic compounds capable of producing the hemolysis of red blood corpuscles. In regard to the cause of the hemolysis after chilling, it might be supposed that its cause could be cryoglobulin [2]. If this specific reaction took place, then the hemoglobin in the plasma would appear only after chilling, but not after physical exertion.

In the light of these facts, we consider the mechanical destruction of erythrocytes in the skeletal muscle blood vessels the cause of the presence of hemoglobin in plasma. We are reinforced in our conviction by the fact that hemoglobin is not found in the plasma during chilling without shivering. On the other hand muscle contraction during work, as well as shivering which is also (tetanically incomplete) contraction, destroys a certain number of old erythrocytes. The rapid uniformity of erythrocyte decrease as soon as after an hour with a simultaneous removal of hemoglobin from the plasma testified to the efficiently acting compensatory mechanisms. The increase in red corpuscle /37
resistance is a proof of this. This fact can only be explained by the eruption of young corpuscles from bone marrow into peripheral blood. We maintain that the increase in resistance is not brought about by a change in the physical properties of the capsules of all the corpuscles, but by increased resistance in young corpuscles.

The brief phase of the drop in the number of erythrocytes makes it difficult to correctly compute the quantitative changes during a single examination, and it can not offer sufficient proof of the mechanical destruction of corpuscles, all the more so because the level of the blood hemoglobin does not vary. Only the finding of hemoglobin present in the plasma indicates a causative connection between this drop and skeletal muscle contraction.

In the light of the results obtained it is easy to explain the constant level of blood hemoglobin after chilling and physical exertion, since we can calculate the amount of both corpuscle and plasma hemoglobin by Sahli's method.

The mechanical destruction of old erythrocytes in the skeletal muscle capillaries is an important factor regulating the renewal of blood corpuscles. The increase in the number of erythrocytes in people participating in sports or working physically is now explained as the result of organism anoxia. The results of our research call attention to another cause, their mechanical destruction. Red corpuscle life is brief in persons of great physical activity. There also exists increased production of red corpuscles in the bone marrow.

Conclusions

1. We found hemoglobin present in the plasma after chilling and physical work. This level was highest immediately after the activity of these factors.

2. After chilling and physical exertion the erythrocytic resistance increased with respect to hypotonic NaCl solutions, probably as the result of an increase in the number of young corpuscles in the blood stream.

3. The number of erythrocytes in veinous blood was reduced by 200,000 to 300,000 in 1 mm^3, and returned to the initial level after one hour.

4. The amount of hemoglobin in veinous blood remained unchanged under the effect of chilling or physical exertion.

5. The cause of the presence of hemoglobin in the plasma and the temporary drop in veinous blood erythrocytes is a mechanical destruction of red corpuscles in the blood vessels of contracting muscles.

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